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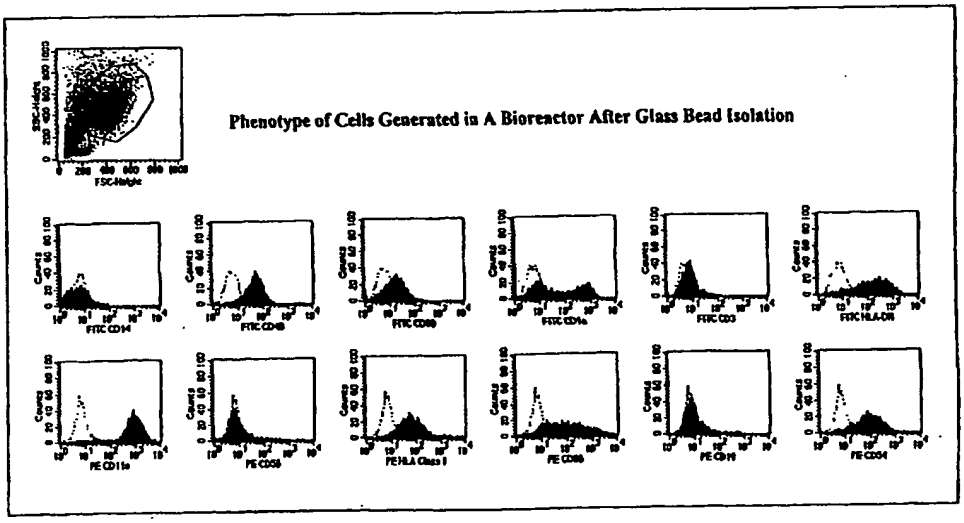
(54) Title: METHODS AND APPARATUS FOR ENRICHMENT AND CULTURE OF MONOCYTIC DENDRITIC CELL PRECURSORS



(57) Abstract: The present invention provides methods and materials for isolating, culturing and differentiating monocytic dendritic cell precursors to form immature and/or mature dendritic cells. In a related aspect, methods, materials, and a system are provided for isolating, culturing and differentiating monocytic dendritic cell precursors under aseptic conditions to obtain immature and/or mature dendritic cells.

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METHODS AND APPARATUS FOR ENRICHMENT AND CULTURE OF MONOCYTIC DENDRITIC CELL PRECURSORS

BACKGROUND OF THE INVENTION

Antigen presenting cells (APC) are important in eliciting an effective immune response. APC not only present antigens to T cells with antigen-specific receptors, but also provide the signals necessary for T cell activation. Such signals remain incompletely defined, but are known to involve a variety of cell surface molecules as well as cytokines or growth factors. The factors necessary for the activation of naive or unprimed T cells may be different from those required for the re-activation of previously primed memory T cells. Although monocytes and B cells have been shown to be competent APC, their antigen presenting capacities appear to be limited to the re-activation of previously sensitized T cells. Hence, they are not capable of directly activating functionally naive or unprimed T cell populations. On the other hand, dendritic cells are capable of both activating naive and previously primed T cells.

Dendritic cells are a heterogeneous cell population with a distinctive morphology and a widespread tissue distribution, including blood. (*See, e.g.*, Steinman, *Ann. Rev. Immunol.* 9:271-96 (1991).) The cell surface of dendritic cells is unusual, with characteristic veil-like projections. Mature dendritic cells are generally identified as $CD86^+$, $CD3^-$, $CD11c^+$, $CD19^-$, $CD14^-$, $CD83^+$ and $HLA\text{-}DR^+$.

Dendritic cells process and present antigens, and stimulate responses from naive and unprimed T-cells and memory T cells. In particular, dendritic cells have a high capacity for sensitizing MHC-restricted T cells and are very effective at presenting antigens to T cells, both self-antigens during T cell development and tolerance, and foreign antigens during an immune response. In addition to their role in antigen presentation, dendritic cells also directly communicate with non-lymph tissue and survey non-lymph tissue for an injury signal (*e.g.*, ischemia, infection, or inflammation) or tumor growth. Once signaled, dendritic cells initiate an immune response by releasing cytokines that stimulate activity of lymphocytes and monocytes.

Due to their effectiveness at antigen presentation, there is growing interest in using dendritic cells as an immunostimulatory agent, both *in vivo* and *ex vivo*. Dendritic cell precursors have been isolated by various methods, such as, for example, density gradient separation, fluorescence activated cell sorting, immunological cell separation techniques such

as panning, complement lysis, rosetting, magnetic cell separation techniques, nylon wool separation, and combinations of such methods. (*See, e.g.*, O'Doherty *et al.*, *J. Exp. Med.* 178:1067-76 (1993); Young and Steinman, *J. Exp. Med.* 171:1315-32 (1990); Freudenthal and Steinman, *Proc. Natl. Acad. Sci. USA* 87:7698-702 (1990); Macatonia *et al.*, *Immunol.* 67:285-89 (1989); Markowicz and Engleman, *J. Clin. Invest.* 85:955-61 (1990).) Methods for immuno-selecting dendritic cells include, for example, using antibodies to cell surface markers associated with dendritic cell precursors, such as anti-CD34 and/or anti-CD14 antibodies coupled to a substrate. (*See, e.g.*, Bernhard *et al.*, *Cancer Res.* 55:1099-104 (1995); Caux *et al.*, *Nature* 360:258-61 (1992).)

The use of such isolated dendritic cells as immunostimulatory agents has been limited, however, due to the low frequency of dendritic cells in peripheral blood and the low purity of dendritic cells isolated by such methods. In particular, the frequency of dendritic cells in human peripheral blood has been estimated at about 0.1% of the white cells. Similarly, there is limited accessibility of dendritic cells from other tissues, such as lymphoid organs.

The low frequency of dendritic cells has increased interest in isolating dendritic cell precursors, and culturing these precursors *ex vivo* to obtain enriched populations of immature or mature dendritic cells. Because the characteristics of dendritic cell precursors are incompletely defined, current methods used for isolating dendritic cell precursors do not result in purified fractions of the desired precursors, but instead generally produce mixed populations of leukocytes. In one example, leukocytes are isolated by a leukapheresis procedure. Additional methods are typically used for further purification to enrich for fractions thought to contain dendritic cells and/or dendritic cell precursors. Similarly, methods such as differential centrifugation (*e.g.*, isolation of a buffy coat) and filtration also produce a crude mixture of leukocytes containing dendritic cell precursors.

Another reported method for isolating dendritic cell precursors is to use a commercially treated plastic substrate (*e.g.*, beads or magnetic beads) to selectively remove adherent monocytes and other "non-dendritic cell precursors." (*See, e.g.*, U.S. Patent Nos. 5,994,126 and 5,851,756.) The adherent monocytes and non-dendritic cell precursors are discarded while the non-adherent cells are retained for *ex vivo* culture and maturation.

In another method, apheresis cells were cultured in plastic culture bags to which plastic, *i.e.*, polystyrene or styrene, microcarrier beads were added to increase the surface area of the bag. The cells were cultured for a period of time for cells to adhere to the beads and the non-adherent cells were washed from the bag. The adherent cells were

cultured in serum free media supplemented with GM-CSF and either IL-4 or IL-7 to provide after about 7 days a population of cells that appeared to be enriched in mature, $CD83^+$, dendritic cells and also contained immature dendritic cells. (Maffei, *et al.*, *Transfusion* 40:1419-1420 (2000); WO 02/44338, incorporated herein by reference)

Typically, once isolated, dendritic cell precursors have been cultured *ex vivo* for differentiation and/or expansion. Briefly, *ex vivo* differentiation typically has involved culturing the mixed cell populations in the presence of cellular growth factors, such as cytokines. For example, granulocyte/monocyte colony-stimulating factor (GM-CSF) and interleukin 4 (IL-4), Interleukin 7 (IL-7), or Interleukin 13 (IL-13) have been used to support and/or differentiate dendritic cells. The numbers of dendritic cells have also been expanded by culture in the presence of cytokines. The effectiveness of such *ex vivo* differentiation and expansion has been limited, however, by the quality of the starting population of dendritic cells. Under some culture conditions, populations of dendritic cells and dendritic cell precursors that are heavily contaminated with neutrophils, macrophages and lymphocytes, or combinations thereof, can be overtaken by the latter cells, resulting in a poor yield of dendritic cells. Culture of dendritic cells containing large numbers of neutrophils, macrophages and lymphocytes, or combinations thereof, are less suitable for use as immunostimulatory preparations.

Once expanded, immature or mature dendritic cells have been exposed to a target antigen(s) to provide activated dendritic cells. In general, the antigen has been added to immature or mature dendritic cells under suitable culture conditions. In the case of immature dendritic cells, the cells are then allowed sufficient time to take up and process the antigen, and express antigenic peptides on the cell surface in association with either MHC class I or class II markers. Antigen can be presented to immature dendritic cells on the surface of cells, in purified form, in a semi-purified form, such as an isolated protein or fusion protein (*e.g.*, a GM-CSF-antigen fusion protein), as a membrane lysate, as a liposome-protein complex, and other methods. In addition, as mature dendritic cells are not capable of taking up and processing antigen, peptides that bind to MHC class I or MHC class II molecules can be added to mature dendritic cells for presentation.

Once activated dendritic cells are obtained, they can be administered to a patient to stimulate an immune response. Activated dendritic cells can be administered to a patient by bolus injection, by continuous infusion, sustained release from implants, or other suitable techniques. The activated dendritic cells also can be co-administered with physiologically acceptable carriers, excipients, buffers and/or diluents. Further, activated

dendritic cells can be used to activate T cells, *e.g.*, cytotoxic T cells, *ex vivo* using methods well known to the skilled artisan.

Most of the above described purification methods share numerous disadvantages, such as being time-consuming, labor-intensive, costly, and/or requiring large amounts of reagents. In addition, the repetitive processing of cells in open systems, which are repeatedly exposed to circulating air or other non-sterile conditions, can be a significant source of contamination. Such contamination can render the isolated cells unsuitable for administration to a subject, such as a human patient. A related disadvantage of such methods is that they can have low specificity, low sensitivity, and/or provide low cell densities of dendritic cells. Thus, large numbers of non-dendritic cells are co-administered in compositions that provide a suitable dose of desired dendritic cells. Finally, many of the purification methods can change the properties, *e.g.*, the number of mature versus immature dendritic cells, the function, or the viability of the resulting dendritic cells.

The cost, quality and effectiveness of antigen-activated dendritic cell populations is of primary concern for immunostimulation of patients. In particular, current methods for dendritic cell culture typically use large amounts of tissue culture media, which requires large amounts of cytokines in the tissue culture media. Due to the cost of cytokines, large tissue culture volumes can substantially add to the cost of preparing antigen-activated dendritic cells. Similarly, antigen-activation of dendritic cells in large media volumes also adds significantly to the cost of preparation. Finally, as discussed above, methods for preparing dendritic cell precursors can be a significant source of contamination. Thus, there is a need for methods of enriching and culturing dendritic cell precursors that are cost effective, allow culture in a small volume, and that reduce the risk of contamination.

BRIEF SUMMARY OF THE INVENTION

The present invention provides methods and materials for isolating, culturing and differentiating monocytic dendritic cell precursors. In one aspect, a method is provided for isolating monocytic dendritic cell precursors from a population of blood leukocytes. The method generally includes contacting a monocytic dendritic cell precursor adhering substrate having a high surface area to volume ratio with the population of leukocytes; allowing the monocytic dendritic cell precursors in the population of leukocytes to reversibly adhere to the substrate to form complexes comprising monocytic dendritic cell precursors and substrate; separating the complexes from the non-adhering leukocytes to obtain enriched complexes comprising monocytic dendritic cell precursors; and culturing the monocytic dendritic cell precursors to differentiate the precursors to form immature or mature dendritic cells. In one

embodiment the monocytic dendritic cell precursors can be eluted from the substrate prior to culturing. Alternatively, the monocytic dendritic cell precursors can be cultured on the substrate. The immature or mature dendritic cells can then optionally be allowed to detach from the substrate as they differentiate.

The population of leukocytes can be prepared, for example, by leukapheresis, density centrifugation, differential lysis, filtration, preparation of a buffy coat, and the like. In one embodiment, the population of leukocytes is substantially free of platelets.

The substrate can have a large surface area to volume ratio of, for example, about 20 square meters per liter to about 80 square meters per liter. The substrate can be, for example, a particulate or fibrous substrate. In certain embodiments, the particulate or fibrous substrate can be microbeads, microcarrier beads, pellets, granules, powder, capillary tubes, microvillous membrane, and the like. In additional embodiments, the particulate or fibrous substrate is glass, polystyrene, plastic, glass-coated polystyrene microbeads, and the like. The particulate or fibrous substrate optionally can have a diameter of about 75 μm and about 300 μm in diameter. In certain embodiments, the substrate is substantially non-porous.

The substrate optionally can be coated with a monocytic dendritic cell precursor-binding protein, with the proviso that the protein is not a monocyte-binding antibody. The monocytic dendritic cell precursor-binding protein can be, for example, granulocyte/macrophage colony stimulating factor (GM-CSF), Interleukin 4 (IL-4), Interleukin 7 (IL-7), Interleukin 13 (IL-13), and the like. In certain embodiments, the surface of the substrate can be treated by acid washing or plasma treatment to enhance monocytic dendritic cell precursor adherence to the substrate.

The method can optionally further include contacting the population of leukocytes and the substrate in the presence of binding media. Suitable binding media can include, for example, protein, plasma, heat inactivated plasma, serum albumin, gamma globulin, divalent cations, DNase, or mixtures thereof. In one embodiment, the population of leukocytes is isolated from a subject and the binding media comprises heat inactivated autologous plasma from the subject. In an alternative embodiment the binding media contains a blocking agent that reduces the amount of cell adhesion to the substrate surface. In particular, the agent increases the specificity of adherence of monocytic dendritic cell precursors to the substrate. The blocking agent can comprise a high concentration of protein, for example, but not limited to serum albumin. A high concentration of protein means any

amount as long as the viability of the cells is not reduced. In a particular embodiment 10 mg/ml of human serum albumin was used.

In another aspect, a method is provided for isolating immature or mature dendritic cells. The method includes contacting a monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio with a population of leukocytes and allowing monocytic dendritic cell precursors in the population of leukocytes to reversibly adhere to the substrate to form complexes of monocytic dendritic cell precursors and substrate. The complexes are separated from the non-adhering leukocytes to obtain enriched complexes of monocytic dendritic cell precursors and substrate. The monocytic dendritic cell precursors can be eluted from the substrate and cultured to differentiate the precursors to immature or mature dendritic cells. In certain embodiments, the monocytic dendritic cell precursors are cultured in the presence of at least one cytokine, such as, for example, GM-CSF, IL-4, IL-7, IL-13, and the like.

In another embodiment, the method further includes contacting the population of leukocytes and the substrate in the presence of binding media. Suitable binding media can include, for example, protein, plasma, heat inactivated plasma, serum albumin, gamma globulin, divalent cations, or a mixture thereof, with the proviso that the binding media does not contain monocyte-binding antibody. The amount of protein that is added to the binding media can be high, as much as at least 1 mg/ml and can be even 100 mg/ml or more, as long as the amount of protein does not decrease the viability of the cells. The method can also optionally include expanding the immature or mature dendritic cells and/or contacting the immature or mature dendritic cells with a pre-determined antigen. Suitable predetermined antigens can include, for example, tumor cells, tumor cell lysate, a purified prostate specific antigen, *i.e.*, but not limitation, prostate specific membrane antigen (PSMA), prostate specific antigen (PSA), prostatic acid phosphatese (PAP), and the like, a tumor-specific antigen, a tumor associated antigen, a bacterial antigen, or a viral antigen, just to name a few. Bacterial and viral antigens can be of any source as the above methods are not limited by the antigen used. The immature or mature dendritic cells can also be contacted with T cells.

In another aspect, a method for preparing immature or mature dendritic cells from a subject is provided. The method generally includes contacting a monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio with a population of leukocytes isolated from a subject; and allowing monocytic dendritic cell precursors in the population of leukocytes to reversibly adhere to the substrate to form complexes of monocytic dendritic cell precursors and substrate. The complexes are then separated from

non-adhering leukocytes to obtain complexes of monocytic dendritic cell precursors. Subsequently, the monocytic dendritic cell precursors are eluted from the surface of the substrate. The monocytic dendritic cell precursors are then cultured to differentiate the precursors, which are contacted with a predetermined antigen. The monocytic dendritic cell precursors can be cultured, for example, in the presence of at least one of, GM-CSF, IL-4, IL-7, IL-13, and the like.

In another aspect, a closed, aseptic system is provided for isolating monocytic dendritic cell precursors from a population of leukocytes. The system typically includes a vessel having a first port and a second port. A monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio is disposed within the vessel; the substrate is in fluid communication with the first port and the second port. At least one screen or filter is disposed within the vessel for retaining the substrate. The screen or filter typically has a pore size sufficient to allow passage of monocytic dendritic cell precursors and dendritic cells therethrough. The vessel can also have a drain line and a collection line in fluid communication with the first port.

The system can optionally include a plurality of fluid sources in fluid communication with the first port and/or the second port, and can also optionally include a source of leukocytes (*e.g.*, leukapheresis material). In certain embodiments, a sealable tissue culture vessel is connected to the vessel to aseptically receive monocytic dendritic cell precursors. Such a sealable tissue culture vessel can be, for example, a tissue culture bag, flask, bioreactor, and the like. The fluid sources can provide, for example, binding media, washing buffer, elution buffer, and the like. The system can optionally include a pump in fluid communication with the fluid sources and the first port. In certain embodiments, the system has a temperature control means, such as a heater, to maintain the substrate at a predetermined temperature.

Another closed aseptic system for isolating monocytic dendritic cell precursors from a population of leukocytes is also provided. The system includes, for example, a vessel having a first port and a second port. A monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio is disposed within the vessel; the substrate is in fluid communication with the first port and the second port. The vessel has a screen or filter for retaining the substrate within the vessel; the screen or filter typically has a pore size sufficient to allow passage of monocytic dendritic cell precursors and dendritic cells therethrough. The vessel typically also includes a drain line and a collection line in fluid communication with the first port. A temperature controlling means can be used to

regulate the temperature within the vessel. A plurality of fluid sources can be connected in fluid communication with the first port or the second port.

The system can optionally include a plurality of valves in fluid communication with the fluid sources and with the first or second port, the plurality of valves regulating the flow of fluid to and from the vessel. In certain embodiments, the valves have a positive position monitor to monitor the valve position. An automated control system can be used to control the plurality of valves.

A pump also can be included in the system. The pump is in fluid communication with the fluid sources and the first port, the pump controlled by the automated control system. The system also can optionally include a temperature control means for controlling the temperature within the vessel. The temperature control means can be, for example, a heater.

The system can optionally be disposed within a cabinet. The cabinet can optionally include a temperature controlled chamber. The vessel can be disposed within the chamber. A source of leukocytes can be connected to the system. A sealable tissue culture vessel can be connected to the system to aseptically receive monocytic dendritic cell precursors. The sealable tissue culture vessel can be, for example, a tissue culture bag, flask, bioreactor, and the like. The fluid sources can be, for example, binding media, washing buffer, elution buffer, and the like. The system can also further include a pump in fluid communication with the fluid sources and the first port.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts flow cytometric analyses of cell surface markers of cultured monocytic dendritic cell precursors cultured on glass beads. The dotted line represents an isotype matched control antibody for the labeled antibody used to detect the specified cell surface molecule.

Figure 2 depicts an exemplary embodiment of a separation device according to one aspect of the present invention.

Figure 3 depicts the characterization of the cell population eluted using the modified monocyte isolation procedures. The characterization was carried out using flow cytometry analysis for the relative abundance of monocytes, as well as the predicted major cellular constituents as determined by the expression of cell surface proteins. Monocytes ($CD14^+$, $CD11c^+$), T cells ($CD3^+$, $CD19^-$), B cells ($CD3^-$, $CD19^+$), NK cells ($CD56^+$, $CD86^-$). The dotted line represents an isotype control antibody for the labeled antibody used to detect the specified cell surface molecule.

Figure 4 depicts a flow cytometric analysis of the cell surface markers on dendritic cells eluted from glass-bead pretreated with a blocking agent comprising a high concentration of human serum albumin.

DESCRIPTION OF THE SPECIFIC EMBODIMENTS

The present invention provides methods and materials for isolating, culturing and differentiating monocytic dendritic cell precursors. Monocytic dendritic cell precursors, such as those from peripheral blood, are typically isolated from a population of leukocytes using a substrate having a high surface area to volume ratio, such as a particulate or fibrous substrate. The monocytic dendritic cell precursors of interest are typically monocytes that selectively adhere to the substrate to form complexes of monocytic dendritic cell precursors and substrate, while other leukocytes remain unbound ("non-adhering"). The complexes are then separated from the unbound leukocytes to form a population of cells enriched in monocytic dendritic cell precursors on the substrate. The monocytic dendritic cell precursors can be cultured and differentiated on the substrate, or eluted from the substrate and then cultured and differentiated, to obtain immature and/or mature, antigen-presenting dendritic cells. It appears that the dendritic cells obtained following culture on the surface of the substrate and those obtained by elution from the substrate prior to culture are functionally different. The population of cells obtained following culture on the substrate, particularly a polystyrene or styrene substrate, comprising more mature ($CD83^+$) dendritic cells.

In a related aspect, methods and materials are provided for isolating, culturing and differentiating monocytic dendritic cell precursors in a closed, aseptic system. Monocytic dendritic cell precursors are isolated or enriched from a population of leukocytes using, for example, the substrate having a high surface area to volume ratio, such as a particulate or fibrous substrate. The monocytic dendritic cell precursors can be cultured and differentiated on the substrate or eluted from the substrate and cultured and differentiated, in the closed, aseptic system, to obtain immature and/or mature dendritic cells. As above, the dendritic cells obtained following culture on the surface of the substrate having a high surface area to volume ratio and those obtained by elution from the substrate prior to culture are functionally different. The population of cells obtained following culture on the substrate, particularly a polystyrene or styrene substrate, comprising more mature ($CD83^+$) dendritic cells.

Dendritic cells are a diverse population of antigen presenting cells found in a variety of lymphoid and non-lymphoid tissues. (*See, e.g.*, Steinman, *Ann. Rev. Immunol.* 9:271-96 (1991).) Dendritic cells include lymphoid dendritic cells of the spleen, Langerhans

cells of the epidermis, and veiled cells in the blood circulation. Collectively, dendritic cells are classified as a group based on their morphology, high levels of surface MHC-class II expression, and absence of certain other surface markers expressed on T cells, B cells, monocytes, and natural killer cells. In particular, mature monocyte-derived dendritic cells usually express CD11c, CD83, are HLA-DR$^+$, but are typically CD14$^-$. In contrast, monocytes are usually CD14$^+$. Monocyte-derived dendritic cells can also express co-stimulatory molecules, such as CD86.

Monocytic dendritic cell precursors, which are typically monocytes, are classified as a group based on their morphology and are usually CD14$^+$. Monocytic dendritic cell precursors can be obtained from any tissue where they reside, particularly lymphoid tissues such as the spleen, bone marrow, lymph nodes and thymus. Monocytic dendritic cell precursors can also be isolated from the circulatory system. Human peripheral blood is a readily accessible source of human monocytic dendritic cell precursors. Umbilical cord blood is another source of human monocytic dendritic cell precursors. Monocytic dendritic cell precursors can also be isolated from a variety of organisms in which an immune response can be elicited. Such organisms include, for example, humans, primates, mammals (including dogs, cats, mice, and rats,) and birds (including chickens), as well as transgenic species thereof.

Isolation of Monocytic Dendritic Cell Precursors

Monocytic dendritic cell precursors are typically isolated from blood by initially enriching for leukocytes. Leukocytes generally include acidophilic, agranular, basophilic, eosinophilic, and neutrophilic leukocytes; lymphocytes; monocytes such as dendritic cell precursors and monocyte precursors; and the like. Populations of leukocytes can be prepared by many methods known to those skilled in the art. Such methods typically include collecting heparinized blood, apheresis or leukapheresis, preparation of buffy coats, rosetting, centrifugation, density gradient centrifugation (*e.g.*, using Ficoll (such as FICOLL-PAQUE®), PERCOLL® (colloidal silica particles (15-30 mm diameter) coated with non-dialyzable polyvinylpyrrolidone (PVP)), sucrose, and the like), differential lysis of non-leukocyte cells, filtration, and the like. A leukocyte population can also be prepared by collecting blood from a subject, defibrinating to remove the platelets and lysing the red blood cells. The population of leukocytes can optionally be enriched for monocytic dendritic cell precursors by, for example, centrifugation through a PERCOLL® gradient.

In certain embodiments, leukocytes are prepared in a closed, aseptic system. As used herein, the terms "closed, aseptic system" or "closed system" refer to a system in

which exposure to non-sterilized, ambient, or circulating air or other non-sterile conditions is minimized or eliminated. Closed systems for isolating monocytic dendritic cell precursors generally exclude density gradient centrifugation in open top tubes, open air transfer of cells, culture of cells in tissue culture plates or unsealed flasks, and the like. In a typical embodiment, the closed system allows aseptic transfer of the leukocytes from an initial collection vessel to the substrate and elution from the substrate to a sealable tissue culture vessel without exposure to non-sterile air.

Leukapheresis is one example of a closed system for isolating leukocytes. Leukapheresis generally involves removing blood from a donor using laminar flow properties to separate mononuclear cells (leukocytes) and platelets from red cells and plasma. The red cells and plasma can be returned to the subject during leukapheresis, while the leukocytes are retained for further processing. Any residual platelets can optionally be removed by low speed centrifugation in phosphate buffered saline. Apheresis is another method for isolating leukocytes that can increase the number of monocytes in relation to the other leukocytic cell types.

Leukocytes can be isolated from a variety of subjects, according to the desired use of the dendritic cells. The subject can be a healthy subject. Alternatively leukocytes can be isolated from a subject in need of immunostimulation, such as, for example, a cancer patient, a patient suffering from a bacterial or viral infection, or other patients for which immunostimulation will be beneficial. Likewise, leukocytes can be isolated from a subject in need of immune suppression, such as, for example, a patient having an autoimmune disorder, (*e.g.*, rheumatoid arthritis, diabetes, and the like). Leukocytes also can be obtained from an HLA-matched healthy individual for administration to an HLA-matched patient in need of immunostimulation. The subject can be administered a composition to stimulating the production of leukocytes, *i.e.*, GM-CSF, prior to collection

To isolate monocytic dendritic cell precursors, the population of leukocytes is contacted with a monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio. When the population of leukocytes is contacted with the substrate, the monocytic dendritic cell precursors in the leukocyte population adhere to the substrate. Other leukocytes (including some other potential dendritic cell precursors) exhibit reduced binding affinity to the substrate, thereby allowing monocytic dendritic cell precursors to be preferentially enriched on the surface of the substrate.

The capacity of the substrate to bind monocytic dendritic cell precursors is generally proportional to the accessible surface area of the substrate. Thus, substrates that

have a larger surface area to volume ratio have the capacity to adhere more monocytic dendritic cell precursors per unit volume than, for example, tissue culture flasks, dishes, and like. The substrate can have a surface area to volume ratio of, for example, from about 20 square meters per liter to about 80 square meters per liter.

The monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio can be, for example, a particulate or fibrous substrate. Suitable particulate substrates include, for example but not by way of limitation, glass particles, plastic particles, glass-coated plastic particles, glass-coated polystyrene particles, and other beads suitable for protein absorption. Suitable fibrous substrates include microcapillary tubes and microvillous membrane. The particulate or fibrous substrate usually allows the adhered monocytic dendritic cell precursors to be eluted without substantially reducing the viability of the adhered cells. In one particular embodiment, the substrate is treated with a blocking agent such that the avidity for the cells, including the monocytic dendritic cell precursors is reduced. The blocking agent can comprise a protein or other compositions known to reduce the binding of a cell to a surface. When the agent is a protein the agent can be present in a solution at a high concentration. By a "high concentration" is meant that the protein can be present in any amount that does not substantially reduce the viability of the monocytic dendritic precursor cells. The agent can also be present at a high concentration is the binding media, for example at least about 1 mg/ml and can be as much as about 100 mg/ml or more. In a specific embodiment of the present invention human serum albumin was present in the binding medium at a concentration of 10 mg/ml and 50 mg/ml.

When the substrate is a particulate or fibrous substrate, the substrate is usually substantially non-porous to facilitate elution of monocytic dendritic cell precursors or dendritic cells from the substrate. A "substantially non-porous" substrate is a substrate in which at least a majority of pores present in the substrate are smaller than the cells to minimize entrapping cells in the substrate.

The density of the substrate is optionally just slightly greater than the density of the binding medium, washing medium and/or tissue culture medium, so that gentle agitation keeps the substrate in suspension while simple means such as sedimentation or centrifugation allows its separation from the medium. In certain embodiments, the substrate has a density of about 1.03 to 1.045 g/ml. It should be noted that monocytes have been previously isolated by adherence and elution from glass beads for metabolic studies. Summers *et al.*, *Brit. J. Haematol.* 30:425-434 (1975).

Typically, the particulate substrate is at least a plurality of separate microbeads. The microbeads can have any suitable shape, including spherical, ellipsoid, rhomboid, cubical, tetrahedral, and the like. The microbeads can be, for example, spheres, pellets, granules or powder. In some embodiments, the particles can have dimensions within the range of about 75 μm to about 300 μm (*e.g.*, for beads, within the range of about 75 μm to about 300 μm in diameter).

Suitable particulate substrates further include microcarriers, which are minute particles for binding and culture of eukaryotic cells. Suitable microcarriers are those that allow the adhered monocytic dendritic cell precursors to be eluted without substantially reducing the viability of the adhered cells (*i.e.*, the cells reversibly adhere). The microcarriers typically have a diameter in the range of about 75 μM to 300 μM, more typically from about 100 μM to about 250 μM, or about 130 μM to about 220 μM. The microcarriers are composed of a material which is non-toxic to monocytic dendritic cell precursors. In one exemplary embodiment, the microcarriers are substantially spherical microbeads with a median particle diameter within the range of about 100 μM to about 250 μM. Suitable microcarriers include glass beads, polystyrene beads, plastic beads, glass-coated plastic or polystyrene beads, and the like. In specific embodiments, suitable particulate substrates include Plastic Plus microcarrier beads (a treated styrene copolymer bead having an electrical charge incorporated in the bead to enhance cell attraction from SoloHill Engineering, Inc.) and HilleX microcarrier beads (a styrene copolymer bead having a trimethylamine core from SoloHill Engineering, Inc.).

The surface of the substrate can optionally be treated to enhance adherence of monocytic dendritic cell precursors to the substrate. The surface of the substrate can be coated with, for example, proteins; cytokines such as, Granulocyte/Macrophage Colony Stimulating Factor, Interleukin 4 and/or Interleukin 13; plasma, such as autologous or allogenic plasma; monocyte-binding proteins, and the like. The substrate is not coated with antibodies, however, that bind to monocytes or to monocytic dendritic cell precursor cell surface markers. In addition, the surface of the substrate can be treated with a blocking agent to decrease cell adherence to increase the efficiency of elution of the monocytic dendritic cell precursors from the substrate and to increase the purity of the precursors isolated from the substrate. The substrate can be pretreated with the blocking agent and/or the blocking agent can be included in the binding media. Typically, the blocking agent is a protein or substance that reduces the binding adherence of cells to the substrate surface. In particular, the

blocking agent is a protein or a mixture of proteins, such as, for example, but not by way of limitation, serum albumin, plasma, and the like. The protein can be present in a high concentration so long as the amount of protein does not effect the viability of the monocytic dendritic precursor cells.

After contacting the population of leukocytes with the monocytic dendritic cell precursor adhering substrate, the monocytic dendritic cell precursors adhere to the substrate to form complexes comprising monocytic dendritic cell precursors on the substrate. Monocytic dendritic cell precursor binding can be monitored, for example, by antibody detection using antibodies specific for cell surface proteins typical of monocytic dendritic precursor cells, such as, for example, anti-CD14 antibodies, by FACS forward and side scatter analysis, and the like. Methods for phenotypic analysis of cell surface markers of dendritic cell precursors and immature and mature dendritic cells are well known in the art. In some embodiments, the leukocyte population is contacted with the substrate for about 5 to about 300 minutes, more typically about 30 to about 120 minutes at about 37°C.

The specificity of the adherence of the monocytic dendritic cell precursors to the substrate can optionally be enhanced by addition of binding media. Suitable binding media include monocytic dendritic cell precursor culture media (*e.g.*, AIM-V®, RPMI 1640, DMEM, X-VIVO 15®, and the like) supplemented with individually or in any combination, for example, cytokines (*e.g.*, Granulocyte/Macrophage Colony Stimulating Factor (GM-CSF), Interleukin 4 (IL-4), Interleukin 7 (IL-7), or Interleukin 13 (IL-13)), blood plasma, serum (*e.g.*, human serum, such as autologous or allogenic sera), purified proteins, such as serum albumin, divalent cations (*e.g.*, calcium or magnesium ions) and other molecules that aid in the specific adherence of monocytic dendritic cell precursors to the substrate, or that prevent adherence of non-monocytic dendritic cell precursors to the substrate. Typically, the amount of protein added to the binding media, for example, plasma or serum albumin, is sufficient to limit nonspecific binding of a cell to the substrate. In some embodiments, the blood plasma or serum is heated-inactivated. The heat inactivated plasma can be autologous or heterologous to the leukocytes. One particular embodiment used 1% heat-inactivated autologous plasma. It has been found however that the addition of high concentrations of protein, for example, but not limitation, up to 10 mg/ml or more of human serum albumin, increases the yield and purity of the monocytic dendritic cell population eluted from the substrate. The amount of protein used can be a substantially saturating amount of protein as long as the viability of the monocytic dendritic precursor cells is not substantially reduced.

Following adherence of monocytic dendritic cell precursors to the substrate, the non-adhering leukocytes are separated from the complexes. Any suitable means can be used to separate the non-adhering cells from the complexes. For example, the mixture of the non-adhering leukocytes and the complexes comprising monocytic dendritic cell precursors can be allowed to settle and the non-adhering leukocytes and media decanted or drained. Alternatively, the mixture can be centrifuged, and the supernatant containing the non-adhering leukocytes is decanted or drained from the pelleted complexes.

The complexes can optionally be washed with a suitable washing buffer to remove non-specifically bound leukocytes. Suitable washing buffers include tissue culture media (*e.g.*, AIM-V®, RPMI 1640, DMEM, X-VIVO 15®, and the like), phosphate buffered saline, Dulbecco's phosphate buffered saline, Hank's Balanced Salt Solution, and the like. The wash solution can be supplemented with amino acids, vitamins, proteins and/or hormones to promote the viability and/or proliferation of the monocytic dendritic cell precursors. The efficacy of washing can be monitored by FACS forward and side scatter analysis of the washing buffer, by staining eluted cells for cell surface markers, and the like. Typically, the complexes are washed several times to remove non-specifically bound leukocytes.

Following separation of the non-adherent cells, the cells adhering to the substrate can be either cultured on the surface of the beads or eluted into a culture container. Elution of the cells from the substrate comprises contacting the substrate cell complexes with an elution buffer. The elution buffer can comprise, for example, but not by way of limitation, a balanced salt solution, such as, Hank's Balanced Salt Solution, a buffered saline solution, such as phosphate buffered saline, with a non-toxic chelating agent, such as EDTA.

In certain embodiments, the monocytic dendritic cell precursor adhering substrate is disposed in a separation device. The surface area to volume ratio of the substrate in the separation device is large relative to the surface area to volume ratio of the separation device alone. For example, a separation device, such as a chromatography column, can have from about 20 square meters to about 80 square meters of bead surface area per liter of volume. The substrate optionally can be retained in the separation device by a screen or mesh filter and/or trapping filter (collectively "screen"). The filters can be disposed in the device above and/or below the substrate.

A port is typically disposed above the top of the substrate bed while another port is typically disposed below the substrate bed. In one embodiment, comprising screens, a port is typically disposed above the substrate and above or below the upper filter while

another port is typically disposed below the bottom screens. Alternatively, the ports can be disposed at any other suitable positions.

To isolate monocytic dendritic precursor cells, a population of leukocytes can be contacted with the substrate by introducing the leukocytes into the column, typically through a column port. The monocytic dendritic precursor cells are allowed to adhere to the substrate. To separate the non-adhering leukocytes from the substrate, the non-adhering cells can be removed by allowing them to drain through a port. Alternatively, the non-adhering cells can be removed with an upward flow of suitable buffer (*e.g.*, tissue culture media, phosphate buffered saline, and the like) followed by draining the buffer and non-adhering cells through a port. This process can optionally be repeated to remove additional non-adhering cells. As will be appreciated by the skilled artisan, under suitable flow conditions, the substrate can assume the properties of a fluidized bed for better removal of non-adhering cells.

In another embodiment, a mixture of leukocytes is contacted with the monocytic dendritic cell precursor adhering particulate or fibrous substrate. The monocytic dendritic cell precursors adhere to the substrate. To separate the non-adhering cells from the complexes, the mixture is added to the separation device. The non-adhering leukocytes can be removed by allowing them to drain through a port. Alternatively, the non-adhering cells can be removed with an upward flow of suitable buffer (*e.g.*, tissue culture media, phosphate buffered saline, and the like) followed by draining the buffer and non-adhering cells through a port, such as an outlet. This process can optionally be repeated to remove additional non-adhering cells. As will be appreciated by the skilled artisan, under suitable flow conditions, the substrate can assume the properties of a fluidized bed for better removal of non-adhering cells.

Following separation of the non-adhering cells from the complexes, the adhered monocytic dendritic cell precursors can be eluted from the substrate. For example, the precursors can be eluted from the substrate by treatment with a balanced salt solution or buffered saline, *i.e.*, phosphate buffered saline, containing 0.4% EDTA or other non-toxic chelating agent. The monocytic dendritic cell precursors are typically eluted from the substrate without the use of an enzyme, such as trypsin, or other proteases. The monocytic dendritic cell precursors are typically eluted in a small volume of from about 10^6 cells/milliliter of elution buffer to about 10^7 cells/milliliter of elution buffer. In some embodiments, the eluted cells comprise at least about 25 %, or at least about 50% monocytic dendritic cell precursors.

In another aspect, a separation device is provided for the aseptic isolation of monocytic dendritic cell precursors. Referring to Figure 2, the separation device is a closed, aseptic system. The system includes a vessel 10. Vessel 10 can have any suitable size and shape. The vessel is composed of any suitable material that can be sterilized or otherwise rendered aseptic.

The vessel can have optionally have a temperature control means to control the temperature within the vessel. Such a temperature control means can include, for example, a heater, such as a forced air heater, an electrical heater, and the like. The temperature control means can also include a cooler, refrigerator or other cooling device. The temperature control means can include, for example, a heater (or heater and cooling device) electrically connected to a temperature sensor and/or a temperature controller (*e.g.*, feedback temperature controller) to control the temperature within the vessel 10. The temperature control means typically maintains a temperature of about 37°C, although other temperatures are possible and within the scope of the invention.

Vessel 10 contains, for example, a particulate or fibrous substrate 15. The substrate 15 is typically disposed between a lower screen, 17 and an upper screen 19. The upper and lower screens have a pore size sufficient to retain the substrate 15 therebetween, but to allow the passage of fluids and cells through the pores of the screen. For example, the pore size can be about 30 to about 50 microns. Alternatively, the substrate 15 is retained in vessel 10 using only an upper or lower screen. The substrate 15 can also be contained in vessel 10 by other means, such as an upper and/or lower screen, or frit disposed over a port.

Vessel 10 can have any suitable arrangement of one or more ports, such as an inlet and/or outlet. Fluids can be introduced into and withdrawn from the vessel 10 through a port 20 and/or port 30. As used herein, the term "port" refers to an aperture that can be an inlet and/or outlet. In one exemplary embodiment, a port 20 can be disposed below lower screen 17. In another exemplary embodiment, a port 30 can be disposed above the upper screen 19. In another exemplary embodiment, a port 31 can be disposed between an upper and/or lower screen.

The introduction of fluids into and out of the vessel 10 optionally can be controlled by one or more valves 23. A valve 23 can be any suitable type of valve, such as a manually controlled valve, an electrically controlled valve, and the like, which provides for and controls fluid communication through the valve 23. The valves in different parts of the system can be the same or different. A valve 23 can be disposed adjacent a port 30 to control the introduction and withdrawal of fluids through that port. Similarly, a valve 23 can be

disposed adjacent port 20, 30 and/or 31 to control the introduction and withdrawal of fluids through that port.

The valve 23 can optionally be controlled electronically, for example, by connection to an automated control system. In one embodiment, a plurality of valves is controlled by the automated control system according to a preset program. The valves can optionally have a positive position monitor to monitor the valve state (*e.g.*, open or closed) and report incorrect valve positions.

A port can be connected to and in fluid communication with a fluid source line 110 and/or fluid sources, such as, for example, sources of tissue culture media, binding media, washing buffer and/or elution buffer. For example, phosphate-buffered saline (PBS-EDTA) can be provided by a first fluid source 50, and phosphate buffered saline (PBS) can be provided by a second fluid source 60. Tissue culture media can be provided by a third fluid source 70, and binding media can be provided by a fourth fluid source 80. In one embodiment, each fluid source can be connected to the port 20, 30 and/or 31 by fluid source line 110. The introduction of fluid from a fluid source can be controlled by valves 23 and/or a pump 40. For example, introduction of fluid from a fluid source into the fluid line 110 can be controlled by activation of the corresponding valve 23 and/or pump 40. In one embodiment, a plurality of valves 23 and the pump 40 are controlled by an automated control system according to a preset program.

A waste line 90 can be connected to and in fluid communication with port 20, 30 and/or 31. Similarly, a collection line 100 can be connected to and in fluid communication with a port 20, 30 and/or 31. The fluid supply line 110, waste line 90 and collection line 100 can be connected to the port in any suitable configuration, as will be appreciated by the skilled artisan.

The system can optionally be disposed in a cabinet 100 to house one or more components of the system. For example, the vessel, fluid sources, valves, lines and pump can be disposed within a cabinet 100. In one embodiment, the cabinet 100 has a temperature controlled chamber enclosing the vessel 10. The temperature controlled chamber can optionally enclose other elements, such as, for example, the valves 23, a valve manifold, the fluid sources, the lines, a pump or pumps 40, and the like. The temperature within the chamber can be maintained by any suitable means, such as, for example, a temperature control means, an electric heater, a force air heater, and the like. The temperature controlled chamber can optionally include one or more temperature sensors electrically connected to, for example, a feedback temperature controller to control the temperature within the chamber. In

addition the system can comprise a device to agitate the vessel during the process. The agitation can comprise a swirling or vortexing of the vessel to suspend the substrate. Agitation of the vessel can be carried out at any point during the process, including after sample introduction, during the wash steps, and during the elution of the monocytic dendritic cell precursors from the substrate. The cabinet and/or chamber can optionally have a means (such as a door or other opening) for accessing the system component(s) disposed therein. Other configurations of the system, including the order and arrangement of valves, lines, ports, pumps and fluid sources, are possible and within the scope of the invention.

In an exemplary embodiment, the system and/or vessel 10, comprising the particulate or fibrous substrate 15 is sterilized or otherwise made aseptic. A binding media is introduced into vessel 10 from source 80 by opening corresponding valve 23 and/or activating pump 40 and opening the valve 23 adjacent the port 20. A population of leukocytes in binding media is introduced into the vessel through port 20 and corresponding valve 23. Alternatively, the population of leukocytes and binding media can be introduced simultaneously into the vessel 10.

The population of leukocytes is contacted to the substrate 15 for a sufficient amount of time for the monocytic dendritic cell precursors to adhere to the substrate. For example, the population of leukocytes can be contacted with the substrate for about an hour, and up to about 18 to 24 hours. The non-adhering leukocytes are then removed through port 30 or by draining through port 20 and drain line 90. The substrate is subsequently washed with washing buffer to remove non-specifically bound leukocytes. For example, the substrate can be washed three times with a washing buffer, such as, for example, tissue culture media (*e.g.*, RPMI containing divalent cations), followed by a wash with phosphate buffered saline. The latter washing buffer can remove divalent cations. As exemplified in Figure 2, tissue culture media can be provided by fluid source 70, through fluid supply line 110 and port 20. After washing, the washing buffer is removed through port 20 and drain line 90. Similarly, PBS washing buffer can be supplied from fluid source 60.

The adhered monocytic dendritic cell precursors can be collected by elution with an elution buffer, such as, for example, Hank's Balanced Salt Solution or phosphate buffered saline containing EDTA or another non-toxic chelating agent. The elution buffer can be provided, for example, by fluid source 50, through supply line 110 and port 20. The elution buffer is introduced into the vessel 10 for a time sufficient to allow the monocytic dendritic cell precursors to disengage from the substrate. The eluted cells in elution buffer can then be collected aseptically in an aseptic tissue culture vessel, such as, for example, a

sealable tissue culture vessel such as a tissue culture bag. Several elutions can optionally be performed, depending on the efficiency of elution of the cells, and the volume of cells to be eluted. The monocytic dendritic cell precursors can then be cultured, expanded, and/or differentiated in the culture vessel, as discussed *infra*. The vessel can be agitated during one or more stages in the process described above. For example, the vessel can be agitated during sample addition, during one or more wash steps, and/or during elution of the monocytic dendritic cell precursors from the substrate.

Culture, Expansion and Differentiation of Monocytic Dendritic Cell Precursors

The eluted monocytic dendritic cell precursors can be cultured in any suitable tissue culture vessel, such as, for example, a tissue culture flask, bag, plate or a bioreactor (including a fermenter). The bioreactor can be equipped with appropriate inlets and/or outlets for introducing the cells, the substrate, sterile gas (*e.g.*, oxygen, carbon dioxide, and/or air), tissue culture media, and the like. The bioreactor will also typically have means for controlling the temperature within it. The bioreactor is typically operated at about 37°C. An outlet can be used to remove cells, substrate and/or media (*e.g.*, tissue culture media, washing media, and the like) from the device. The bioreactor can also have means for agitating the culture medium and substrate in the bioreactor. The agitation means can include, for example, a paddle or a spin filter (which also can function as an outlet for media). The substrate and the cells can be kept in suspension by the agitation means.

In an exemplary embodiment, the monocytic dendritic cell precursors are cultured in a closed, aseptic system such as a bioreactor, tissue culture bag, and the like. As used herein, the terms "closed system" or "closed aseptic system" refers to a system in which exposure of the monocytic dendritic cell precursors to non-sterile conditions (*e.g.*, non-sterile ambient air) is minimized. By contrast, an open system includes a tissue culture flask or plate for which the cap or cover is left open, or is unsealed, to provide air exchange with non-sterile ambient air. The closed system can, however, have an inlet and/or outlet for the controlled, aseptic introduction of fluids (*e.g.*, tissue culture media, washing buffer), gases, cells, and the like.

In one embodiment, the monocytic dendritic cell precursors can be isolated on a substrate, eluted from the substrate, and transferred to a bioreactor, or other closed system, such as a tissue culture bag. Suitable tissue culture bags include, for example, STERICELL® culture containers (Nexell Therapeutics Inc.) or TEFLON® culture bags (American Fluoroseal Corp.). The closed system can have any suitable size or volume, as will be appreciated by the skilled artisan. Suitable volumes include, for example, from about 0.01

liters to about 5 liters, or about 0.01 liters to about 0.05 liters, although greater and lesser volumes are possible and within the scope of the present invention.

The monocytic dendritic cell precursors can also be cultured on the substrate. For example, the monocytic dendritic cell precursors on the substrate can be cultured in a bioreactor (including a fermenter) or tissue culture vessels, such as tissue culture flasks, bags, or plates. The tissue culture flasks, bags or plates can have any suitable size or volume, as will be appreciated by the skilled artisan. A bioreactor typically has a volume of from about 0.01 to about 5 liters, or about 0.01 to about 0.05 liters, although greater and lesser volumes are possible and within the scope of the present invention. Typically, the bioreactor used for culture of monocytic dendritic cell precursors will have a volume of about 0.01 to about 0.1 liters. The bioreactor can be inoculated with any suitable amount of monocytic dendritic cell precursors, such as, for example, from about 10^5 cells to about 5×10^6 cells per milliliter of substrate. The monocytic dendritic cell precursors on the substrate can also be cultured in a closed, aseptic system.

The monocytic dendritic cell precursors are cultured and differentiated to obtain immature or mature dendritic cells. Suitable tissue culture media include AIM-V®, RPMI 1640, DMEM, X-VIVO 15®, and the like. The tissue culture medium can be supplemented with amino acids, vitamins, cytokines, such as granulocyte/macrophage colony stimulating factor (GM-CSF) and/or interleukin 4 (IL-4), interleukin 7 (IL-7) or interleukin 13 (IL-13), divalent cations, and the like, to promote differentiation of the monocytic dendritic cell precursors to immature dendritic cells. A typical cytokine combination is about 500 units/ml each of GM-CSF and IL-4. Typically, if the monocytic dendritic cell precursors are cultured on the substrate the number of mature dendritic cells recovered as the cells unadhere from the substrate surface are primarily mature dendritic cells.

The monocytic dendritic cell precursors can be cultured for any suitable time. In certain embodiments, suitable culture times for the differentiation of precursors to immature dendritic cells can be about 4 to about 7 days. The differentiation of immature dendritic cells from the precursors can be monitored by methods known to those skilled in the art, such as by monitoring the presence or absence of cell surface markers, such as, CD14, CD11c, CD83, CD86, HLA-DR, with labeled monoclonal antibody. The phenotype of the dendritic cells can also be determined by analysis of patterns of gene expression by methods well known in the art. A typical cell surface phenotype for immature dendritic cells would be $CD14^-$, $CD11c^+$, $CD83^{lo}$, $CD86^-$, and $HLA\text{-}DR^+$. Immature dendritic cells can also be cultured in appropriate tissue culture medium to expand the cell population and/or maintain

the immature dendritic cells in a state for further differentiation or antigen uptake, processing and presentation. For example, immature dendritic cells can be maintained and/or expanded in the presence of GM-CSF and IL-4.

Immature dendritic cells can be preferred for optimal antigen presentation because they retain the ability to process new antigen. (*See, e.g.*, Koch *et al.*, *J. Immunol.* 155: 93-100 (1995).) In contrast, mature dendritic cells (*e.g.*, $CD14^-$, $CD11c^+$, $CD83^+$, $CD86^+$, $HLA\text{-}DR^+$), those that have been exposed to and process antigen and to suitable maturation agents, have typically lost the ability to efficiently process new antigens. Mature dendritic cells can be contacted with peptides that are capable of binding to MHC class I and/or MHC class II molecules for presentation on the cell surface.

During culture, immature dendritic cells can optionally be exposed to a predetermined antigen. Suitable predetermined antigens can include any antigen for which T-cell activation is desired. In one embodiment, immature dendritic cells are cultured in the presence of a prostate antigen, such as prostate specific membrane antigen (PSMA), prostate specific antigen (PSA), prostatic acid phosphatase (PAP), and the like, for cancer immunotherapy and/or tumor growth inhibition. Other antigens can include, for example, bacterial and viral antigens, tumor cells, purified tumor cell membrane, tumor cell lysate, tumor specific or tumor associated antigens (*e.g.*, isolated antigens from tumors, fusion proteins, liposomes, and the like), bacterial cells, bacterial antigen, viral particles, viral antigens, and any other antigen. In addition, an antigen can be expressed on the surface of a transformed or transfected host cell expressing the antigen, or a purified membrane or a cell lysate of a tranfected or transformed cell expressing the antigen.

Following contacting with antigen, the cells can be cultured for any suitable time to allow antigen uptake and processing, to expand the population of antigen-specific dendritic cells, and the like. Immature dendritic cells can also be matured into mature dendritic cells that present antigen in the context of MHC class I or MHC class II molecules. Such maturation can be performed, for example, by culture in the presence of known maturation factors, such as cytokines (*e.g.*, TNF-α), CD40 ligand, bacterial products (*e.g.*, bacillus Calmette-Guerrin (BCG)), and the like.

According to another aspect, dendritic cells exposed to a predetermined antigen can be used to activate T cells *in vitro* against an antigen. The dendritic cells can be used immediately after exposure to antigen to stimulate T cells. Alternatively, dendritic cells can be maintained in the presence of a combination of cytokine (*e.g.*, GM-CSF and IL-4)

prior to exposure to antigen and T cells, or the dendritic cells can be cryopreserved by methods well known in the art for use at a later time. In a specific embodiment, human dendritic cells are used to stimulate human T cells.

T cells or a subset of T cells can be obtained from various lymphoid tissues for use as responder cells. Such tissues include but are not limited to the spleen, lymph nodes, and peripheral blood. T cells can be co-cultured with dendritic cells exposed to the predetermined antigen as a mixed T cell population or as a purified T cell subset.

For example, purified $CD8^+$ T cells can be co-cultured with antigen-exposed dendritic cells to elicit an antigen-specific CTL. In addition, early elimination of $CD4^+$ T cells can prevent the overgrowth of $CD4^+$ cells in a mixed culture of both $CD8^+$ and $CD4^+$ T cells. T cell purification can be achieved by positive or negative selection including, but not limited to, the use of antibodies directed to CD2, CD3, CD4, CD5, and/or CD8. Alternatively, mixed populations of $CD4^+$ and $CD8^+$ T cells can be co-cultured with dendritic cells to elicit a response specific to an antigen encompassing both a cytotoxic and T_H immune response.

EXAMPLES

The following examples are provided merely as illustrative of various aspects of the invention and shall not be construed to limit the invention in any way.

Example 1: Isolation of Monocytes on Glass Beads

To isolate monocytes from a population of leukocytes, leukapheresis material (comprising primarily leukocytes and some other cellular material) was first washed twice using low-speed centrifugation with phosphate buffered saline (PBS) to remove platelets and plasma. The resulting cell pellet (1.8×10^9 cells) was resuspended in a total volume of 10 ml AIM-V® media (Gibco-Life Science) containing 1% heat-inactivated autologous plasma (binding media). Glass beads, about 125 to about 212 μm in diameter, (20 grams) were prepared by washing twice in binding media and were subsequently placed in a 60 milliliter syringe fitted with a frit to retain the beads to form a column bed. The binding media was then drained from the column bed by gravity flow. The leukapheresis material was applied to the column, and any flowthrough was collected. Binding media was added to provide a small layer of liquid above the column bed. The column with cells was then incubated at 37°C for 30 minutes.

After incubation, the column port was opened, and the flowthrough was collected. The column bed was then washed six times with binding media (35 ml/wash)

administered and removed multiple times through the column port to allow gentle resuspension of the beads. These washes were followed by two washes with phosphate buffered saline ("PBS"). Cell counts were obtained for all washes and the original flow through, and they were analyzed by forward and side scatter FACS analysis to determine the percentage of monocytes present. After completing the washes, the bound monocytes were eluted from the beads using PBS/0.4% EDTA (w/v), followed by one more PBS wash. The cells obtained in these fractions were analyzed in the same manner as the washes.

The cell counts and per cent monocytes in the flowthrough, washes and elutions are presented below in Table 1. FACS analysis was not done ("n.d.") for the flowthrough and for washes 1-6, as the relatively large number of red blood cells in these fractions prohibited such analysis.

Table 1

Analysis of Monocytes Bound to Glass Beads

Fraction	Media	Total cell count	Monocytes
Flow-through	Binding media	2.0×10^8	n.d.
Wash 1	Binding media	2.8×10^8	n.d.
Wash 2	Binding media	3.3×10^8	n.d.
Wash 3	Binding media	1.4×10^8	n.d.
Wash 4	Binding media	2.2×10^8	n.d.
Wash 5	Binding media	7.5×10^7	n.d.
Wash 6	Binding media	4.2×10^7	n.d.
Wash 7	PBS	2.1×10^7	2.3×10^6 (11.1)
Wash 8	PBS	3.1×10^7	9.8×10^6 (31.5)
Wash 9	PBS	6.8×10^7	4.0×10^7 (58.9)
Elute 1	PBS/0.4% EDTA	1.4×10^8	1.1×10^8 (72.9)
Elute 2	PBS	5.6×10^7	4.0×10^7 (71.9)
Elute 3	PBS	4.0×10^7	2.9×10^7 (73.5)

From this table, it can be seen that monocytes preferentially adhere and are retained by glass beads. Large numbers of monocytes are retained by the glass beads through wash 8. In contrast, other cells either do not adhere (flowthrough) or adhere weakly and are more readily eluted from the glass beads with PBS (washes 1-8). Large numbers of monocytes were eluted in wash 9 and elution 1 (using a PBS/EDTA step). The combined total number of monocytes present in wash 9 and elution steps 1-3 (*i.e.*, excluding cells from washes 7 and 8) was 2.1×10^8, representing 12% of the starting cell count in the leukapheresis material with an overall purity of 70%. Additional elutions after elution 3 did not contain significant numbers of cells.

Example 2: Formation of Dendritic Cells From Monocytes Eluted From Glass Beads

The eluted monocytes from Example 1 were washed two times with 30 mls of PBS, and resuspended in culture media (X-VIVO 15® (BioWhittaker Corp.) with 500 U GM-CSF/ml and 500 U/ml IL-4. A portion (approximately 2/3) of the cell suspension was then transferred to a rotary bioreactor (Synthecon) and cultured for 6 days at 37°C in a humidified environment containing 5% CO_2. After culturing, the cell population was analyzed and found to contain 7.2×10^7 immature dendritic cells out of 9.9×10^7 cells total, a purity of

72%. The immature dendritic cells were considered to be typical based on cell size and granularity, and on cell surface marker expression.

Example 3: Monocyte Isolation and Dendritic Cell Culture on Glass Beads

Approximately 5 x 10^8 cryopreserved PBMC were passed through a column containing 2.5 grams of glass beads, followed by 3 washes with X-VIVO 15® tissue culture media (BioWhitaker). Evaluation of the column flow-through and washes showed that approximately 49% of the initial starting number of cells were still attached to the glass beads. Furthermore, flow cytometric scatter analyses showed that the beads retained >70% of the monocytes present in the initial cell population. The glass beads were resuspended in 50 ml dendritic cell culture media (X-VIVO 15® with 500 U/ml GM-CSF and 500 U/ml IL-4), transferred into a bioreactor and cultured for 6 days. After six days in the bioreactor, the total number of dendritic cells harvested was 5 x 10^7 cells (a 10% yield) with 3.6 x 10^7 lymphocytes counted (about 58% dendritic cell purity). Flow cytometric analyses of dendritic cell markers confirm the identity ($CD14^-$, $CD11c^+$; see Figure 1) of the dendritic cells. The purity obtained using this method of preparation can be further improved upon using washing techniques that allow mild resuspension of the beads, as described above in Example 1.

Example 4: Differentiation of Monocytic Dendritic Cell Precursor Following Capture on Plastic Beads

Leukocytes obtained from leukapheresis material were absorbed to a column of plastic beads having a trimethylamine core (HilleX, SoloHill Engineering Inc.). After washing, the beads were resuspended in tissue culture media (X-VIVO-15®, BioWhittaker) and cultured in a flask in the presence of GM-CSF and IL-4 (500 U/ml each). The resulting cells were harvested and evaluated for cell marker phenotype with labeled monoclonal antibodies as described above and FACS analysis. Based on the forward and side scatter profiles, typical dendritic cells were obtained. Analysis of cell surface marker expression confirmed this identification.

Example 5: Adherence Binding of Monocytes to Microcarrier Beads

In this example, the effect of microcarrier beads on monocytes was examined. In particular, it was determined whether incubation of peripheral blood monocytes ("PBMC") with microcarrier beads would deplete cells with the characteristics of monocytes from the cell mixture. PBMC were incubated with two different types of microcarrier beads at 37°C, and after 1 hour of incubation the beads were separated from the unbound cells. The

unbound cells were then analyzed by flow cytometry. The following table shows representative results using Plastic Plus microcarrier beads (a treated styrene copolymer bead haivng an electrical charge incorporated in the bead to enhance cell attraction) from SoloHill Engineering Inc.) and HilleX microcarrier beads (SoloHill Engineering Inc.).

Table 2

Percentage monocytes in PBMC before and after incubation with microcarrier beads

Beads	% monocytes in PBMC	% monocytes in unbound fraction
Plastic Plus	20.47	7.57
HilleX	20.47	5.11

As can be seen from Table 2, both types of microcarrier beads caused depletion of monocytes from the cell mixture.

Example 6: Generation of Dendritic Cells from Cells Bound to Microcarrier Beads

Various microcarrier beads were added directly to leukapheresis material, after that material had been substantially depleted of platelets using low-speed centrifugation. The beads with bound monocytes were then put in tissue culture media containing GM-CSF and Interleukin 4 (500 U/ml each) for 6 days without eluting the cells from the substrate. After incubation, the cells were analyzed by flow cytometry for size, morphology, and for the expression of cell-surface markers. Table 3 lists the total number of cells and the percentage of cells with immature dendritic cell morphology from bead adherent cells.

Table 3

Yield of Cells from Different Types of Beads

Beads (type)	Yield (x 10^6)	Purity (visual)	Purity (FACS)
HilleX	5.4	60%	37%
Glass	5.5	75%	60%
Plastic Plus	19.8	25%	20%
None*	9	56%	60%

* indicates dendritic cells obtained via conventional means.

Table 4 show the expression of cell surface markers on dendritic cells obtained from bead-adherent cells.

Table 4

Cell Surface Markers on Cultured Cells Isolated from Microcarrier Beads

Beads (type)	$CD14^+$	DR^+	$CD86^+$	$CD1a^+$	$CD83^+$
Hillex	0.13	92%	65%	45%	60%
Glass	0.53	90%	91%	0.2%	10%
Plastic Plus	0.10	90%	95%	4%	32%
None*	0.36	74%	90%	9%	7%

* indicates DC obtained via conventional means.

The data in Tables 3 and 4 demonstrate that cells that are being selected based on their adherence to microcarrier beads can give rise to immature and mature dendritic cells.

Example 7: Activation of Immature Dendritic Cells with Prostate Specific Antigen

Monocytic dendritic cell precursors are isolated from a prostate cancer patient as described in Example 6. The precursors are eluted from the microcarrier beads and cultured in tissue culture bags in X-VIVO 15® (BioWhittaker) tissue culture media supplemented with GM-CSF and Interleukin 4 (500 U/ml each) for 6 days at 37°C. The resulting immature dendritic cells are then exposed to prostate specific antigen (PSMA) (isolated as described in U.S. Patent No. 5,788,963, incorporated herein by reference) added to the culture media. The immature dendritic cells are then differentiated to form mature dendritic cells using a maturation agent. The mature (activated) dendritic cells are added to a T cell proliferation assay. T cell cultures are incubated in a humidified 37°C incubator supplemented with 5% CO_2 for about 5 days prior to addition of 1 μCi ^{3}H-Thymidine/well of a microtiter plate. After a 24 hour incubation, the cells are harvested in a semi-automatic cell harvester (Skatron, Stevina, Va.), and the radioactivity of the collected cells is determined. T cell proliferation is assessed by measurement of average ^{3}H-TdR incorporation.

Example 8: Capacity of Cultured Mature Dendritic Cells to Present Antigen

To assess the capacity of the cultured, mature dendritic cells to present antigen to and stimulate autologous T cells from the same patients, T cell proliferation assays are conducted as described above in Example 7. Tetanus toxoid is chosen as the representative antigen in these experiments to determine whether patients' memory T cells can be activated *in vitro*. Autologous T cells cultured with the patient's dendritic cells and Tetanus toxoid will proliferate at levels significantly higher than background levels (in the absence dendritic cells) and at levels significantly higher than T cells cultured with mature (activated) dendritic

cells without Tetanus toxoid (*i.e.*, showing an autologous mixed lymphocyte reaction). Thus, the presentation of Tetanus toxoid by dendritic cells is useful for T cell proliferation.

Example 9: Administration of Dendritic Cells to a Subject

The ability of mature (activated) dendritic cells to present antigen specific for a desired antigen, for example, prostate cancer and to stimulate autologous T cells of a prostate cancer patient is determined. A crude cellular lysate of LNCaP cells, a metastatic prostate cancer cell line that expresses PSMA, is used as a representative prostate cancer antigen in a T cell proliferation assay generally as described in U.S. Patent No. 5,788,963 (the disclosure of which is incorporated by reference herein). A significant increase in ^{3}HTdR incorporation is observed when both mature activated dendritic cells and LNCAP lysates are included in the T cell cultures.

Example 10: Isolation of Monocytes with Human Serum Albumin (HSA)-Coated Glass Beads.

The recovery of isolated monocytes with HSA-coated glass beads was compared to an identical procedure with non HSA-coated beads as described in Example 1 above. Briefly, peripheral blood mononuclear cells (approximately 2.5-2.9 x 10^{9}) were resuspended in 4 ml column binding media (AIM-V® medium (Gibco-Life Science); 10 mg/ml HSA (Bayer); 30 U/ml DNAse I (Sigma)). These cell suspensions were loaded on to separate columns. The first column contained 15 grams of glass beads (about 125 -212 μm, SoloHill Engineering Inc.). The second column contained 15 grams of glass beads that had been pre-incubated overnight at 37°C with a solution containing 50 mg/ml HSA in phosphate buffered saline (PBS). The cells were incubated in the column for 60 minutes. After incubation, the columns were washed (50 ml/wash) twice with binding media, followed by washing three times with AIM-V®, and once with Hanks-Balance Salt Solution (HBSS (BioWhittaker)). Each wash was introduced from the bottom of the column at a rate of 2.5 ml/min, to allow for gentle resuspension of the beads. After completing the washes, the bound monocytes were eluted from the beads using HBSS/0.4% EDTA (w/v). Comparison of the recovery of monocytes from the two columns are presented in Table 5.

Table 5

Monocyte Isolation Using Glass Beads or HSA-Coated Glass Beads

Glass Beads	Start PBMC (x 10^{8})	Start Monocytes in PBMC (x 10^{8})	Monocytes Eluted (x 10^{8})	Monocyte Purity (%)	Monocyte Recovery (%)
Non-coated	25.4	5.6	2.4	54.3	42.9

HSA-coated	29.7	5.8	2.5	74.7	43.1

From this table, it can be seen that the purification experiment using HSA-coated beads resulted in significant increase in purity (74.7% from 54.3%), compared to a similar procedure with non-coated glass beads.

Example 11: Isolation of Monocytes with Glass Bead Column using An Automated Separation Device.

Monocyte isolation procedures with glass bead column were also conducted using a separation device as depicted in Figure 2. These procedures were performed both manually and in an automated manner under software control.

Each run utilized the following base protocol:

1. Pre-incubating the beads with 50 mg/ml Human Serum Albumin (HSA)
2. Warming the device to 37°C and assembling the setup with media bags, column and device
3. Washing and equilibrating the beads with binding media
4. Preparing the sample
5. Introducing and incubating the sample
6. Washing:
   a. Four times with Binding Media
   b. Two times with Wash Media 1
   c. Once with Wash Media 2
7. Eluting:
   a. Wash media 2
   b. Twice with Elution Media
8. Washing and buffer exchange for culture and cryopreservation

The source of starting material in this example was cryopreserved PBMC isolated from leukapheresis material by HISTOPAQUE®(Sigma-Aldrich, St Louis, MO) density gradient centrifugation. The number of PBMC applied to the bead column in these experiments ranged from about 36×10^8 to about 68×10^8 cells.

Binding Media and Wash Media 1 tested in these experiments was X-VIVO15® (BioWhittaker, Walkersville, MD). The Binding Media contained 10 mg/ml HSA and 30 U/ml DNAse I. The Wash Media 1 contained no additional supplements. The Wash Media 2 was phosphate buffered saline (PBS) (Mediatech, Walkersville, MD). The Elution

Media was 0.4% EDTA in phosphate buffered saline (PBS). Samples were introduced for 6 min at 2 ml/minute. Wash speeds were 8 ml/min. Elution speeds were 15 ml/min.

A summary of the data obtained using the various isolation procedures conducted under these conditions in both manual and automated controls are presented in the following table.

Table 6

Monocyte Isolation in Using Glass Bead Columns in a Separation Device

Run Mode	Start PBMC ($x10^8$)	Start Monocytes in PBMC ($x10^8$)	Monocytes Eluted ($x10^8$)	Monocyte Purity (%)	Monocyte Recovery (%)
Manual	46.0	12.7	4.6	72.7	36.1
Manual	36.0	13.3	4.1	69.4	30.9
Manual	58.0	23.0	5.3	71.6	23.0
Automated	63.0	22.2	5.1	70.1	23.0
Automated	68.0	23.5	9.3	74.3	39.6

Monocyte purities of 69.4% to 74.3% (average = 71.6%) in the final column eluant, were achieved. Monocyte recoveries of 23.0% to 39.6% (average = 30.5%) were achieved. Relative monocyte abundance in the eluted populations were estimated from the forward and side scatter properties, as analyzed using flow cytometry. The first three experiments were conducted manually. The next two experiments were conducted with the automated mode. No significant difference in monocyte purity or recovery was observed between the manual and automated runs. Total time spent in conducting each procedure was 5-6 hours.

Further analysis of the eluted cell populations were carried out by flow cytometry. The relative abundence of monocytes as well as the major cellular contaminants, *e.g.*, T cells, B cells and NK cells, were determined. Monocytes ($CD14^+$, $CD11c^+$ cells) were found to make up the majority of the eluted cell population (78.6 %), while T cells, B cells and NK cells were found at a low percentage ranging from about 2.2 % to abount 3.0 %.

The above isolation procedure was then modified to see of the monocyte isolation procedure could be improved. The modifications included the following: (i) increasing the sample volume to 65 ml; (ii) changing the sample introduction rate to 1 ml/minute for 58 minutes; (iii) addition of column swirling action that helps bead resuspension at certain time periods including: (a) after sample introduction; (b) during the

three washes in binding media during the elution step with PBS/0.4% EDTA. Each of these changes in the isolation procedure resulted in significant improvements in monocyte purity and recovery in the eluted population. Data from these experiments are presented in the following table.

Table 7

Monocyte Isolation in Using Glass Bead Columns in a Separation Device

Run Mode	Start PBMC ($x10^8$)	Start Monocytes in PBMC ($x10^8$)	Monocytes Eluted ($x10^8$)	Monocyte Purity (%)	Monocyte Recovery (%)
Manual	60.0	26.2	8.0	84.9	30.4
Manual	67.8	26.6	16.0	85.3	62.0
Automated	58.0	17.8	7.0	86.7	39.3

Monocyte purities of 84.9% to 86.7% (average = 85.6%) in the final column eluant, were achieved. Monocyte recoveries of 30.4% to 62.0% (average = 43.9%) were achieved. Significant improvements in both purity (85.6% vs. 71.6%) and recovery (42.9% vs. 30.5%) were demonstrated when these modifications were added to the previous procedure.

Example 12: Generation of Dendritic Cells (DCs) from Precursors Eluted from Glass Beads.

Cell populations eluted from glass bead columns were washed twice with XVIVO-15® (BioWhittaker), and resuspended in X-VIVO-15® medium (BioWhittaker) containing 2 % human serum albumin (HSA, Bayer); 500 U/ml GM-CSF (Immunex) and 500 U/ml IL-4 (R&D Systems). Cell suspensions were transferred into tissue culture flasks (Greiner) or cell culture bags (American Fluoroseal Corporation), and incubated in a 37°C, 6 % CO_2 incubator for 3 to 6 days. At the conclusion of the culture period, maturation factors (1:400 dilution of inactivated bacillus Calmette-Guerrin (BCG), and 500 U/ml interferon-γ (IFN-γ) were added to the culture. DCs were matured for 18 to 24 h in a 37°C, 6 % CO_2 incubator, harvested and characterized.

Figure 4 demonstrates that DCs generated from glass bead-eluted monocytic dendritc cell precursors in the presence of a high concentration of human serum albumin express typical DC markers, including CD11c, CD1a, CD80, CD86 and HLA-DR. The lack of CD14 expression was used as a demonstration that the cells had converted from monocytes ($CD14^+$) into DCs. In addition, a fraction of the cell population demonstrated CD83 expression, a typical marker for mature DCs. The extent of cell viability was measured by

propidium iodide staining using standard procedures. The purity of live DCs in this population was 88.1 % ($CD11c^+$, propidium $iodide^-$ cell population). The percentage of viable cells within the DC population was 93.8 %.

The previous examples are provided to illustrate but not to limit the scope of the claimed inventions. Other variants of the inventions will be readily apparent to those of ordinary skill in the art and encompassed by the appended claims. All publications, patents, patent applications and other references cited herein and are also incorporated by reference herein.

WHAT IS CLAIMED IS:

1. A method of isolating monocytic dendritic cell precursors from a population of blood leukocytes, comprising:

contacting the population of leukocytes with a monocytic dendritic cell precursor adhering substrate having a high surface area to volume ratio and pretreated with a non-specific binding blocking agent;

allowing the monocytic dendritic cell precursors in the population of leukocytes to reversibly adhere to the substrate to form complexes comprising monocytic dendritic cell precursors and substrate;

separating the complexes from the non-adhering leukocytes to obtain enriched complexes comprising monocytic dendritic cell precursors.

2. The method of claim 1, wherein the method further comprises the step of culturing the monocytic dendritic cell precursors for a sufficient time to differentiate the precursors to form immature or mature dendritic cells.

3. The method of claim 1, wherein the nonspecific binding blocking agent is a protein or a mixture of protein.

4. The method of claim 3, wherein the protein is human serum albumin.

5. The method of claim 1, wherein the monocytic dendritic cell precursors are eluted from the substrate prior to culturing.

6. The method of claim 1, wherein the monocytic dendritic cell precursors are cultured on the substrate.

7. The method of claim 6, wherein the mature dendritic cells detach from the substrate.

8. The method of claim 1, further comprising preparing the population of leukocytes by leukapheresis, apheresis, density centrifugation, differential lysis, filtration, or preparation of a buffy coat.

9. The method of claim 1, wherein the population of leukocytes is substantially free of platelets.

10. The method of claim 1, wherein the substrate having a large surface area to volume ratio is a particulate or fibrous substrate.

11. The method of claim 10, wherein the particulate or fibrous substrate comprises microbeads, microcarrier beads, pellets, granules, capillary tubes or microvillous membrane.

12. The method of claim 1, wherein the substrate has a surface area to volume ratio of about 20 square meters per liter to about 80 square meters per liter.

13. The method of claim 10, wherein the particulate or fibrous substrate comprises glass, polystyrene, plastic or glass-coated polystyrene microbeads.

14. The method of claim 10, wherein the particulate or fibrous substrate is between about 75 μm and about 300 μm in diameter.

15. The method of claim 1, wherein the substrate is substantially non-porous.

16. The method of claim 1, wherein the substrate is coated with monocytic dendritic cell precursor-binding protein, with the proviso that the protein is not a monocyte-binding antibody.

17. The method of claim 16, wherein the monocytic dendritic cell precursor-binding protein is granulocyte/macrophage colony stimulating factor, Interleukin 4, Interleukin 7, or Interleukin 13.

18. The method of claim 1, wherein a surface of the substrate is treated by acid washing or protein treatment to enhance monocytic dendritic cell precursor adherence to the substrate or elution from the substrate.

19. The method of claim 1, further comprising contacting the population of leukocytes and the substrate in the presence of binding media.

20. The method of claim 19, wherein the binding media comprises protein, plasma, heat inactivated plasma, serum albumin, gamma globulin, divalent cations, DNase, or mixtures thereof.

21. The method of claim 19, wherein the population of leukocytes is isolated from a subject and the binding media comprises heat inactivated autologous plasma from the subject.

22. The method of claim 20, wherein the serum albumin is human serum albumin.

23. The method of claim 22, wherein the human serum albumin is present at a concentration of at least about 1 mg/ml.

24. A method for isolating immature or mature dendritic cells, comprising:

contacting a monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio with a population of leukocytes;

allowing monocytic dendritic cell precursors in the population of leukocytes to reversibly adhere to the substrate to form complexes comprising monocytic dendritic cell precursors;

separating the complexes from the non-adhering leukocytes to obtain enriched complexes comprising monocytic dendritic cell precursors;

eluting the monocytic dendritic cell precursors from the substrate; and

culturing the monocytic dendritic cell precursors to differentiate the precursors to immature or mature dendritic cells.

25. The method of claim 24, wherein the monocytic dendritic cell precursors are cultured in the presence of at least one cytokine.

26. The method of claim 25, wherein the cytokine is GM-CSF, Interleukin 4, Interleukin 7, or Interleukin 13.

27. The method of claim 24, further comprising contacting the population of leukocytes and the substrate in the presence of binding media.

28. The method of claim 27, wherein the binding media comprises protein, plasma, heat inactivated plasma, serum albumin, gamma globulin, divalent cations, or a mixture thereof, with the proviso that the binding media does not contain monocyte binding antibody.

29. The method of claim 28, wherein the serum albumin is human serum albumin.

30. The method of claim 29, wherein the human serum albumin is present in a concentration of at least from about 1 mg/ml.

31. The method of claim 24, further comprising expanding the immature or mature dendritic cells.

32. The method of claim 24, further comprising contacting the immature or mature dendritic cells with a pre-determined antigen.

33. The method of claim 32, wherein the pre-determined antigen is a prostate specific antigen, a tumor-specific antigen, a tumor associated antigen, a bacterial antigen, or a viral antigen.

34. The method of claim 32, further comprising contacting the dendritic cells of claim 33 with T cells.

35. A method for preparing immature or mature dendritic cells, comprising:

contacting a monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio, wherein the substrate is not styrene or polystyrene, with a population of leukocytes isolated from a subject;

allowing monocytic dendritic cell precursors in the population of leukocytes to reversibly adhere to the substrate to form complexes comprising monocytic dendritic cell precursors and substrate;

separating the complexes from non-adhering leukocytes to obtain complexes comprising monocytic dendritic cell precursors;

culturing the monocytic dendritic cell precursors to differentiate the precursors to form immature or mature dendritic cells; and

contacting the immature or mature dendritic cells with a predetermined antigen.

36. The method of claim 35, wherein the monocytic dendritic cell precursors are cultured in the presence of at least one cytokine selected from the group consisting of GM-CSF, Interleukin 4, Interleukin 7, and Interleukin 13.

37. A closed, aseptic system for isolating monocytic dendritic cell precursors from a population of leukocytes, comprising:

a vessel having a first port and a second port;

a monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio and pretreated with a non-specifc binding blocking agent disposed within the vessel, the substrate in fluid communication with the first port and the second port;

a screen for retaining the substrate within the vessel, the screen having a pore size sufficient to allow passage of monocytic dendritic cell precursors and dendritic cells therethrough;

a drain line in fluid communication with the first port; and

a collection line in fluid communication with the first port.

38. The system of claim 37, further comprising a plurality of fluid sources in fluid communication with the first port or the second port.

39. The system of claim 37, further comprising a source of leukocytes.

40. The system of claim 37, further comprising a sealable tissue culture vessel adapted to aseptically receive the monocytic dendritic cell precursors.

41. The system of claim 40, wherein the sealable tissue culture vessel is a tissue culture bag, flask or bioreactor.

42. The system of claim 37, wherein the fluid sources comprise binding media, washing buffer and elution buffer.

43. The system of claim 37, further comprising a pump in fluid communication with the plurality of fluid sources and the first port.

44. The system of claim 37, further comprising:

temperature control means to maintain the substrate at a predetermined temperature.

45. The system of claim 44, wherein the temperature controlling means is a heater.

46. The system of claim , wherein the system further comprises a means for agitating the substrate in the vessel.

47. A closed, aseptic system for isolating monocytic dendritic cell precursors from a population of leukocytes, comprising:

a vessel having a first port and a second port;

a monocytic dendritic cell precursor adhering substrate having a large surface area to volume ratio disposed within the vessel, the substrate in fluid communication with the first port and the second port;

a screen for retaining the substrate within the vessel, the screen having a pore size sufficient to allow passage of monocytic dendritic cell precursors and dendritic cells therethrough;

a drain line in fluid communication with the first port;

a collection line in fluid communication with the first port;

temperature control means to regulate the temperature within the vessel; a means for agitating the substrate within the vessel; and

a plurality of fluid sources in fluid communication with the first port or the second port.

48. The system of claim 47, further comprising a plurality of valves in fluid communication with the plurality of fluid sources and with the first or second port, the plurality of valves regulating the flow of fluid to and from the vessel.

49. The system of claim 48, wherein the valves have a positive position monitor to monitor the valve position.

50. The system of claim 48, further comprising an automated control system for controlling the plurality of valves.

51. The system of claim 50, further comprising a pump in fluid communication with the plurality of fluid sources and the first port, the pump controlled by the automated control system.

52. The system of claim 47, wherein the temperature control means is a heater.

53. The system of claim 47, further comprising a cabinet, the system disposed within the cabinet.

54. The system of claim 53, the cabinet comprising a temperature controlled chamber, the vessel disposed within the chamber.

55. The system of claim 47, further comprising a source of leukocytes.

56. The system of claim 47, further comprising a sealable tissue culture vessel adapted to aseptically receive the monocytic dendritic cell precursors.

57. The system of claim 56, wherein the sealable tissue culture vessel is a tissue culture bag, flask or bioreactor.

58. The system of claim 47, wherein the fluid sources comprise binding media, washing buffer and elution buffer.

59. The system of claim 47, further comprising a pump in fluid communication with the plurality of fluid sources and the first port.

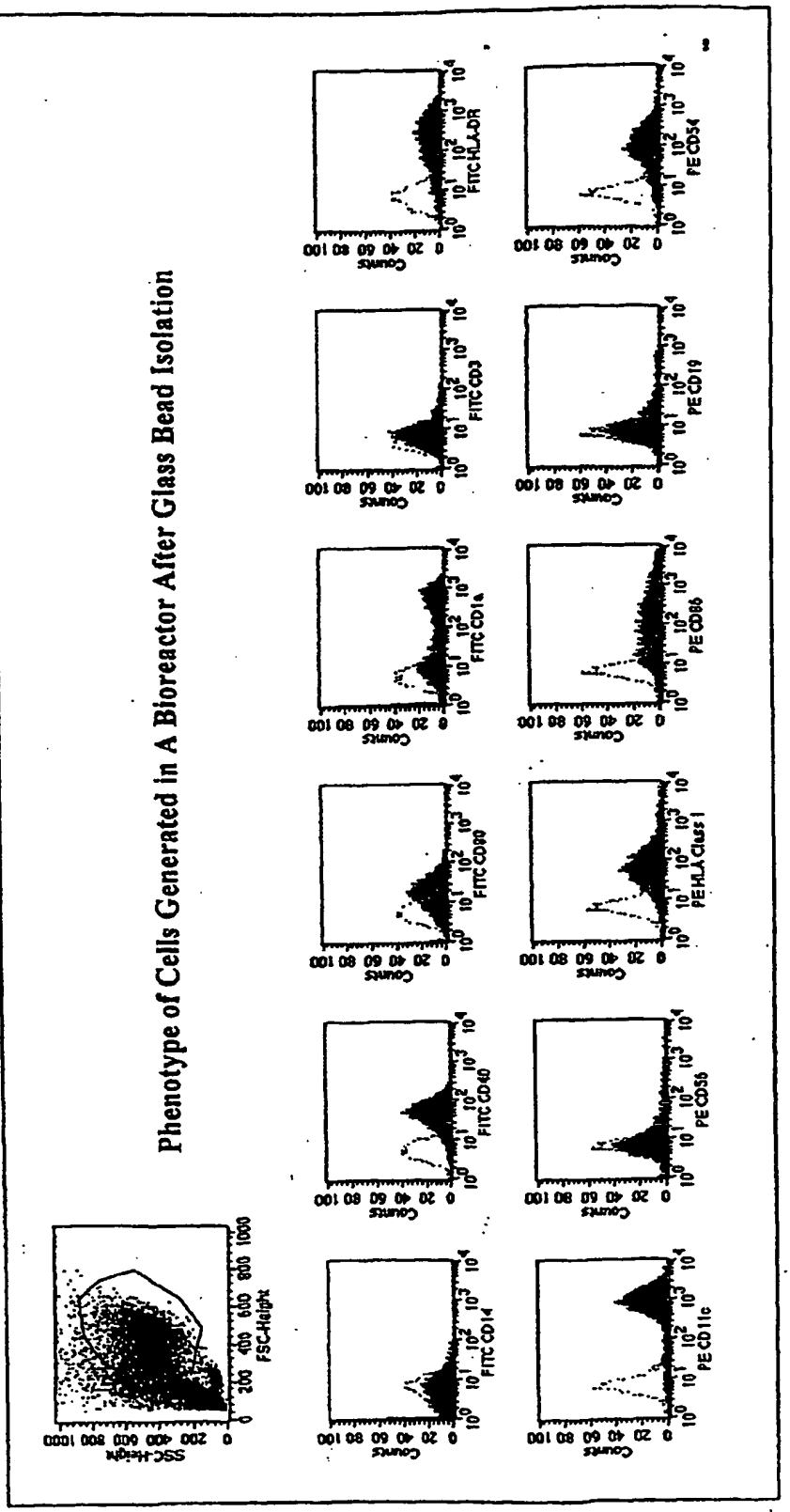
Phenotype of Cells Generated in A Bioreactor After Glass Bead Isolation
SSC-Height
FSC-Height
FITC HLA-DR
PE CD54
FITC CD3
PE CD19
FITC CD14
PE CD86
FITC CD80
PE HLA Class I
FITC CD40
PE CD56
FITC CD14
PE CD11c
Counts

Figure 1

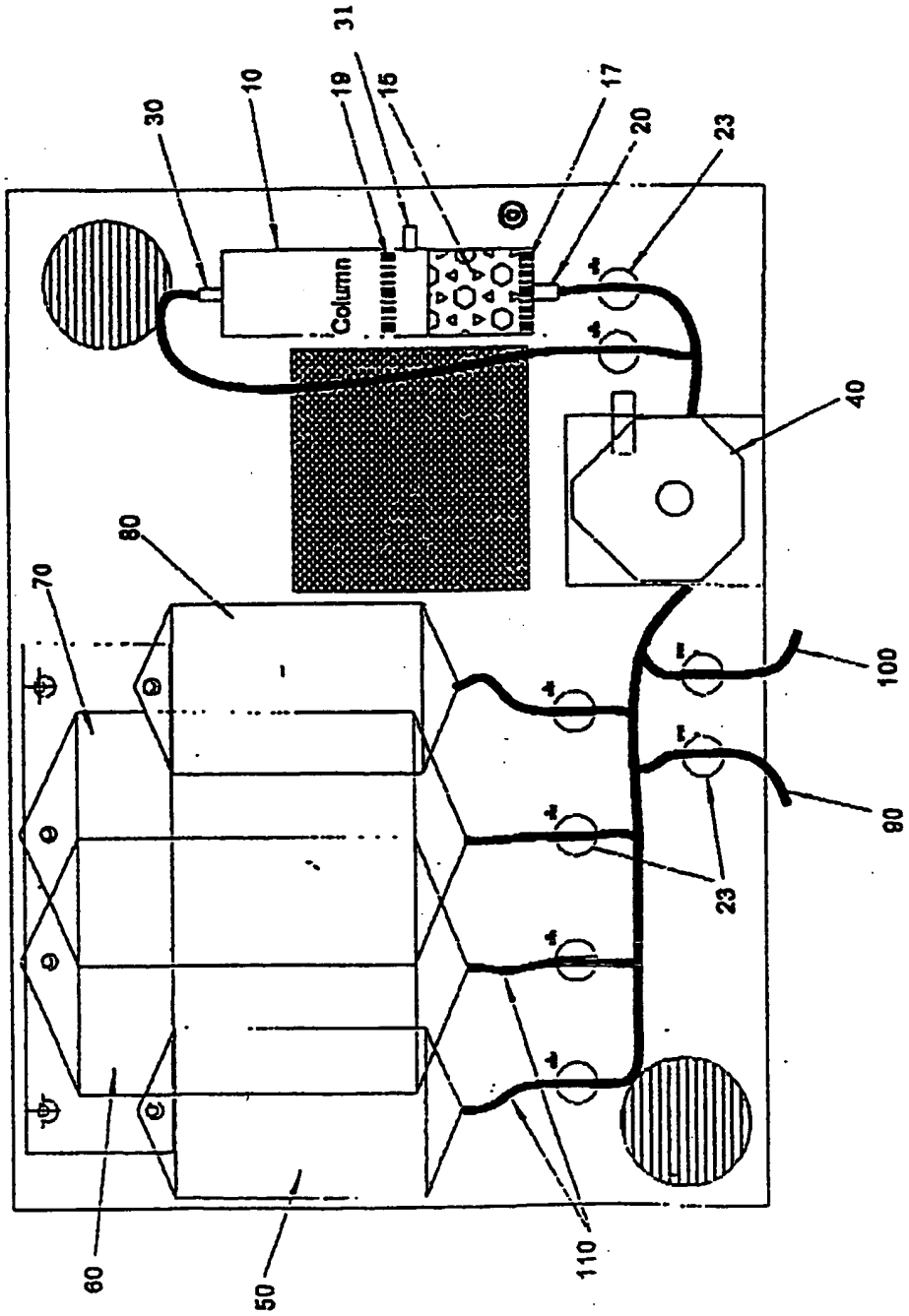

Figure 2

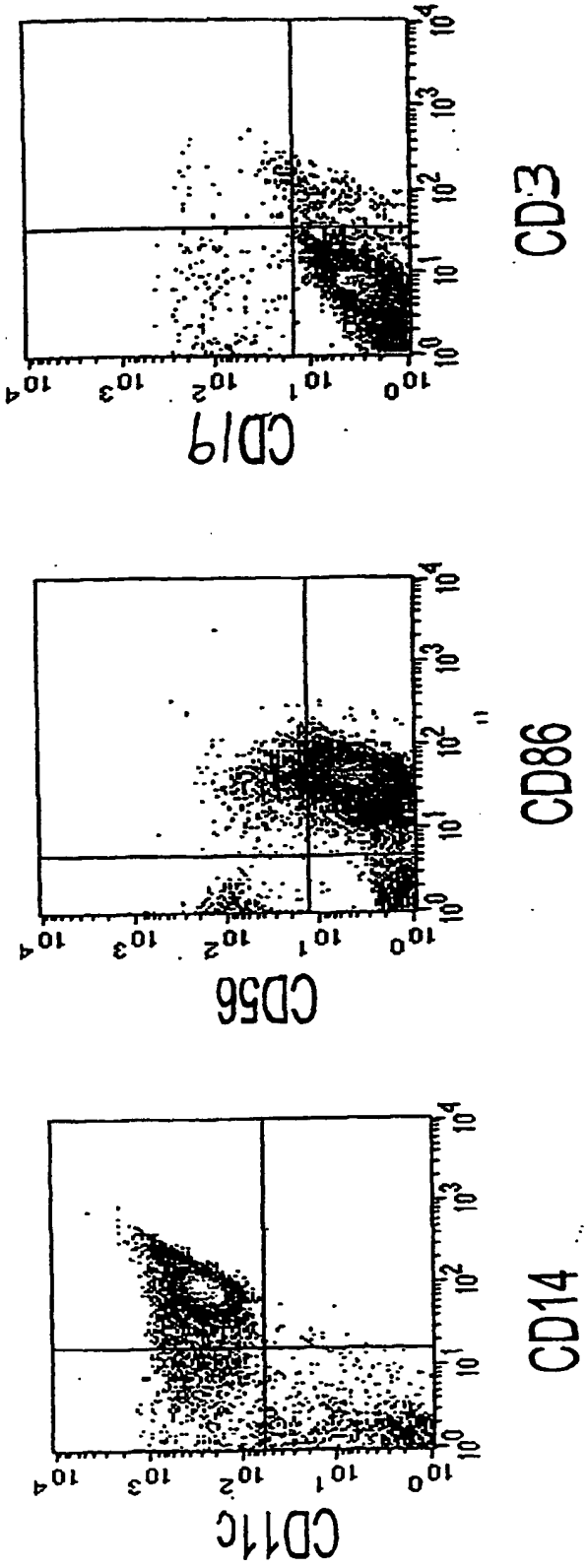

Figure 3

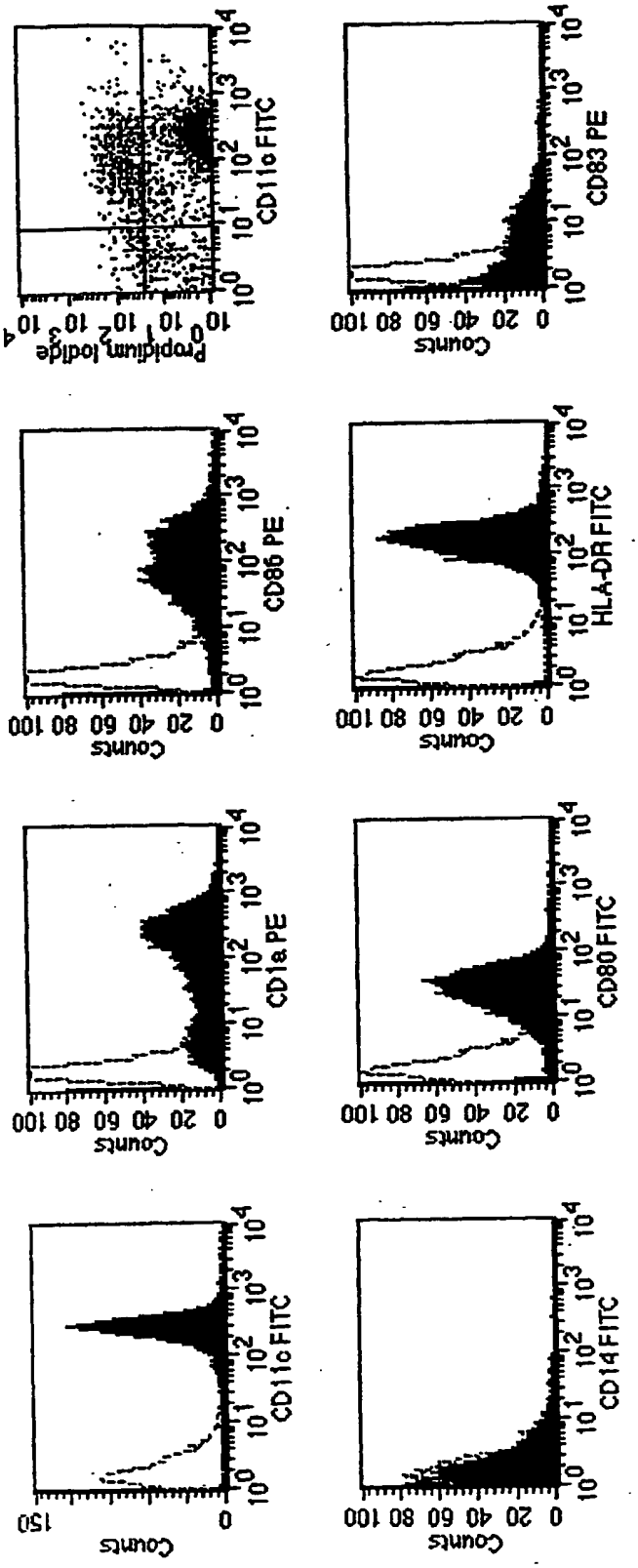
Propidium Iodide
CD11c FITC
Counts
CD83 PE
CD86 PE
HLA-DR FITC
CD1a PE
CD80 FITC
CD11c FITC
CD14 FITC

Figure 4